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# BIOLOGICAL BULLETIN

## THE RELATIVE PERMEABILITY OF THE SURFACE AND INTERIOR PORTIONS OF THE CYTOPLASM OF ANIMAL AND PLANT CELLS.[1]

(A PRELIMINARY PAPER.)

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The permeability and osmotic properties of cells are explained on the assumption of the presence of surface and vacuolar plasmatic membranes which are of a semi-permeable or partially permeable character. It is generally held that the remainder of the protoplasm of a given cell plays a negligible rôle in both diosmosis and permeability. These assumptions are based on indirect evidence. The permeability of the internal cytoplasm of living animal and plant cells has not been investigated by direct methods.

This paper gives the results of a study of the permeability of the internal cytoplasm and nucleus to dyes and crystalloids.

The animal material used in this investigation included the eggs of *Asterias*, *Cumingia*, *Chætopterus*, *Nereis* and the immature eggs of *Necturus*, *Ameba proteus*, *Paramecium* and the striped muscle and epidermal cells of *Necturus*. The plants selected were *Saccharomyces*, *Mucor*, *Saprolegnia*, some five species of *Spirogyra*, *Hydrodictyon*, the manubrial cells of *Chara*, the leaves of *Elodea*, root-hairs of *Vicia faba*, *Pisum*, *Hordeum* and the parenchyma cells of *Tradescantia*.

[1] The studies on marine eggs reported in this paper were carried on during the summer of 1912 at the Marine Biological Laboratory, Woods Hole, Mass., while occupying a table through the courtesy of the Director, Dr. F. R. Lillie, to whom I am indebted for many kindnesses.

A number of methods were used in order to make the data comparative and to determine the error and limitations of a given method employed. The effect of various operations, such as partial dissections, and punctures, on the permeability and osmotic properties of cells was studied. Intracellular injections were made by Barber's method.[1]

## The Permeability of Animal and Plant Cells to Acid Dyes.

Incidental to this study, a number of facts bearing on Overton's so-called lipoid theory have been determined, and as animal physiologists have taken kindly to this notion some of these data will be given.

Rhuland[2] and others have shown a number of exceptions to Overton's[3] conclusion that cells are impermeable to lipoid insoluble acid dyes.

By using a large number of species of animals and plants from widely separated genera and phyla, I have found it quite easy to discover cells that are freely permeable to many lipoid-insoluble acid dyes. Such well-known acid dyes as eosin and trypan red are good vital stains for *Mucor* and *Saprolegnia*.

The lipoid-insoluble acid dyes used include:

Trypan blue, which penetrates the eggs of *Nereis* and *Chætopterus*, the root-hairs of barley and the Windsor bean, immature *Necturus* eggs, the peritoneal epithelium of *Necturus*, *Ameba proteus*, *Paramecium*, *Mucor*, and *Saprolegnia*.

Trypan red: the eggs of *Cumingia* and *Chætopterus*, the root-hairs of barley, the edible pea and the Windsor bean, *Ameba proteus*, *Paramecium*, *Mucor*, and *Saprolegnia*.

Isamin blue: the root-hairs of barley and the Windsor bean.

Analine blue: the eggs of *Chætopterus*, the root-hairs of barley and the Windsor bean, and *Mucor*.

Acid fuchsin: The root-hairs of barley, the Windsor bean and *Mucor*.

Acid green: *Saprolegnia* and *Mucor*.

[1] Barber, *Jour. of Inf. Dis.*, 1911, VIII., p. 348.

[2] *Jahr. Wiss. Bot.*, Bd. 51, H. 3, p. 376.

[3] *Jahr. f. Wiss. Bot.*, Bd. 34, 1900, p. 669.

Acid violet: Windsor bean root-hairs, *Saprolegnia*, and *Mucor*.

Beibricher scarlet: Barley root-hairs, the immature eggs of *Necturus*, the peritoneal epithelium of *Necturus*, *Mucor*, *Saprolegnia*, and *Paramecium*.

Indigo-carmin: *Saprolegnia* and *Mucor*.

Ponceau, P. R.: the root-hairs of the Windsor bean, *Ameba proteus*, *Paramecium*, *Saprolegnia*, and *Mucor*.

Indulin: root-hairs of Windsor bean, *Ameba proteus*, *Saprolegnia*, and *Mucor*.

Nigrosin: the peritoneal epithelium and the very immature eggs of *Necturus*, and *Mucor*.

Eosin: Windsor bean root-hairs, *Ameba proteus*, *Paramecium Mucor*, and *Saprolegnia*.

In recent papers Loewe[1] states that even basic dyes are adsorbed by lipoids, when added to solutions of lipoids in organic solvents.

THE INTRACELLULAR INJECTION OF DYES AND CRYSTALLOIDS.

If indigo-carmin, methyl red, trypan blue, thiocarmine R., or azolitmin dissolved in sea-water be injected into any portion of the cytoplasm of the starfish egg, the sea water slowly diffuses into the surrounding protoplasmic gel, and finally the granular precipitated dye alone remains. An injection of indigo-carmin into the cytoplasm of an immature egg of *Necturus* results in a slight staining of the wall of the vacuole, while an injection of such widely different dyes, as indigo-carmin, trypan blue, and janus green (diethyl-safranin-azo-dimethyl-anilin), into the cytoplasm of the striped muscle cell of this animal results at most in a localized staining of the cytoplasmic gel immediately surrounding the mass of injected dye. The injection of indigo-carmin or nigrosin into the vacuole of *Spirogyra* results in a blue-green or light violet staining, respectively, of the entire cytoplasm. A blue-green staining of the cytoplasm of *Hydrodictyon* is affected by an intravacuolar injection of indigo-carmin. In fact, every acid dye that was injected into the vacuole of *Spirogyra*, *Hydrodictyon*, the leaf cells of *Elodea*, and the parenchyma cells of *Tradescantia* penetrated, and stained the cytoplasm and usually the nucleus.

[1] Loewe, *Biochemische Zeitschrift*, 1912, XLII., p. 150.

Particularly striking permeability phenomena are to be observed when dyes that do not penetrate *Ameba proteus* are injected into the interior. Azolitmin, congo red, tropeolin ooo No. 1, sodium alizarin sulphonate, and indigo-carmin were used for these injections.

All the dyes enumerated were dissolved in salt and sugar solutions of different concentrations, and small and large doses were injected. It was found that these dyes diffuse quickly through the interior of *A. proteus*, if the concentration of the salt or sugar solution be not too high. A localized blue vacuole results when indigo-carmin, dissolved in distilled water, is injected into the ectoplasm of this animal. Usually in a short time the vacuole breaks into the interior and the dye rapidly diffuses.

Sea water, distilled water, solutions of sodium chloride, potassium nitrate, and cane sugar have been injected into the interior of different types of cells. A small dose of distilled water is taken up by the surrounding cytoplasm of the starfish egg quite slowly. A vacuole of sea water requires a somewhat longer time to disappear, while a vacuole filled with hypertonic sea water increases in size. Hence, any portion of the cytoplasm of the starfish egg can exhibit the same general diosmotic properties that are shown by the surface. It seems that this is also true for the cytoplasm of the striped muscle cell of *Necturus*, but on account of the very high viscosity of this substance the wall surrounding the injected fluid remains very irregular, and no accurate measurement of the volume injected could be made. Distilled water is absorbed from the interior of the muscle substance extremely slowly; and salt and sugar solutions more slowly or not at all.

Doses of 1 m. sodium chloride and potassium nitrate and from .5 M. to 2 M. cane sugar diffuse through the cytoplasm of *Ameba proteus* when injected into the interior. The vacuoles that are formed by the injected fluid quickly collapse. Granules, fibrils, and globules can be produced in *proteus* by the injection of 2 M. cane sugar. The shrunken part of the cytoplasm does not readily take up water again.

In this connection it may be added that the injection of mercury and isotonic salt solutions and the introduction of various

foreign bodies into protoplasm usually lead to remarkably little reaction.

## The Effect of Chemical Treatment and Operations on the Permeability of Cells.

Early in this investigation a general relation between permeability and the degree of concentration of protoplasmic gels was noted. Several methods were devised for grading the concentration of the surface and interior portions of the cytoplasm of various cells. The desired concentration gradient can be produced in the eggs of *Asterias*, *Chætopterus*, *Cumingia*, and *Nereis* either by treating with very dilute acids, alkalies and saponin dissolved in sea water or puncturing or cutting with extremely fine Jena glass needles. It was proved that such operative treatment does not kill any portion of the egg.

If the egg of *Asterias* be punctured, the acid dyes used penetrate the swollen area for varying depths, but never enter the normal unswollen cytoplasm. Under the conditions of my experiments only the swollen surface was penetrated and stained. It cannot be overemphasized that the concentration gradient experiment has proved an adequate test of the correctness of the membrane conception, at least for the eggs of *Asterias*, *Cumingia*, *Chætopterus* and *Nereis*. Dyes were selected which do not penetrate the normal egg. These dyes penetrate the swollen cytoplasm produced by the operation or chemical treatment, to varying depths, but never enter the unswollen cytoplasm. Some of the acid dyes do not penetrate the surface of the swollen area of a punctured egg, while others are stopped only by the unchanged cytoplasm. These results are exactly the converse of what would necessarily follow if the plasmatic membrane conception were really true. Moreover, it was noted that trypan red and erythrosin stain the surface of a normal *Chætopterus* egg, but even a great increase in concentration of the dyes does not produce more than a surface staining. Here the surface is actually more permeable to these dyes than the interior of the egg. Again, the nucleus of the eggs of *Asterias*, *Cumingia*, *Chætopterus*, and *Nereis* remain unstained when the eggs are placed in the best vital stains in use at the present time. Under this set of condi-

tions the nuclear membrane is impermeable to vital stains. This structure is a concentrated tough gel of relatively high viscosity and is not to be confused with hypothetical surface or vacuolar plasmatic membranes. If the concentration of a suitable vital stain be raised considerably or the nucleus be dissected out, staining occurs.

Puncture of the walls and probably the cytoplasm of various types of plant cells has given unanticipated results. All the more common acid dyes enter the cells of such plants as *Spirogyra*, *Elodea*, *Hydrodictyon*, different root-hairs, and the parenchyma cells of *Tradescantia*, following an extremely small puncture. Puncture of the walls of slightly plasmolyzed *Spirogyra* and *Elodea* cells is followed by protoplasmic staining when acid dyes are added. On the other hand, puncture of thoroughly plasmolyzed *Spirogyra* cells is followed at most by a slight surface staining, of the shrunken and concentrated cytoplasm, by the same acid dyes.

During plasmolysis of *Spirogyra* a large amount of mucilaginous material is poured out of its wall, and, as a result, this structure loses much of its rigidity, becomes softer, and permeable to many acid dyes. This striking change can be demonstrated by vital stains, dissection of the wall of thoroughly plasmolyzed cells, and by staining cells immediately after recovery from thorough plasmolysis.

A very small cut in the wall of a manubrial cell of *Chara* gives even with trypan blue and trypan red only a local staining of the cytoplasm, which slowly spreads.

## The Dissection of Cells.

The chief cells that have formed the material for this study have been dissected by the use of adequate methods, and their physical properties, such as rigidity, viscosity, glutinicity, elasticity, tenacity, and colloidal state, have been determined. Although these data are too extensive to be given here, they form a part of the basis of my conclusions.

## Conclusions.

1. The structural components of protoplasm vary greatly in their permeability to water, dyes, and crystalloids.

2. Impermeability or partial permeability to water, dyes, and crystalloids is a property of all portions of protoplasmic gels.

3. The rate of penetration of protoplasm by dyes and crystalloids is, in general, inversely proportional to the concentration of the living gel.

4. The best vital stains known penetrate such highly concentrated protoplasm as the epithelial and striped muscle cells of *Necturus* very slowly.

5. The interior portions of the cytoplasm of the starfish egg and probably the striped muscle cell of *Necturus* exhibit the same sort of osmotic properties as the surface.

6. The cell-walls, and not the protoplasm of many plant cells, prevent the entrance of dyes.